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Poly(*N*-isopropylacrylamide-*co*-*N*-vinylpyrrolidone) as a Novel Implant Materials: Preparation and Thermo-Gelling Behavior[†]

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Abstract : Synthesis of polymers with controlled thermosensitive properties was carried out by conventional radical copolymerization of *N*-isopropylacrylamide (NIPAAm) with *N*-vinylpyrrolidone (NVP) taken as a hydrophilic comonomer. Lower activity of NVP rather than NIPAAm was revealed by gravimetric and ^{1}H NMR analysis. Thermosensitive properties of the copolymers were investigated. It was found that aqueous solutions of the copolymers undergo thermo-induced phase transition and become opaque, precipitate or gel with heating. After formation of the gels their significant contraction was observed at storage. Swelling degree and amount of expelled water were measured in dependence on the copolymer composition, temperature and ionic strength of environment medium and concentration of the solution. It was determined that in collapsed state gels exhibit quite high water content. According to physico-chemical properties of the copolymers observed they could be suitable for biomedical application as an injectable implant material.

Keywords : NIPAAm-NVP copolymers, implant materials, thermo-gelling.

Introduction

Thermal phase transition from a polymer aqueous solution to a hydrogel is found at decreasing or increasing temperature for various kinds of natural[1-3] and synthetic polymers.[4-11] First type of phase transition takes place due to strengthened hydrogen bonds between macrochains rather than with water molecules at cooling. It is common for many natural polysaccharides and proteins such as gelatin, collagen, and chitosan. Second one is relatively rare, and occurred also as hydrogen bonds of polymer chains with water weaken and hydrophobic interactions in polymer are involved. However, the mechanism of thermo-induced gelation is not clearly understood yet. At present the accepted explanation is temperature dependent conformation transition from random coil to compact globule, micelle formation and then their packing, resulting in loss of micelles mobility. This thermally reversible gelation upon heating (inversive gelation) is only reported for methylcellulose, some poly(ethylene oxide)-*block*-poly(propylene oxide) copolymers.[3,4]

Among synthetic polymers characterized by thermo-gelling behavior, poly(*N*-isopropylacrylamide) (polyNIPAAm) is the most interesting and the most developed. It exhibits lower critical solution temperature (LCST) at around 32 °C in distilled water, and precipitates or gels above this temperature.[4,11] Copolymerization of NIPAAm with hydrophobic or hydrophilic comonomers, addition of salt, cosolvent, surfactant[7] and other species allows manipulating by LCST value and gelling behavior. For instance various hydrophilic and hydrophobic substances such as acrylic acid,[12] acrylamide,[13] butylmethacrylate,[12,14] *N*-methacryloyl *L*-lucine,[11] 2-(dimethylamino) ethyl methacrylate,[14,16] ethylene glycol dimethacrylate,[4] polyvinylalcohol[17] were taken as possible comonomers. Thus, increase of hydrophilicity degree at the presence of hydrophilic units such as acrylic acid and acrylamide in NIPAAm-based polymers results in increase of LCST value. In contrast copolymerization with hydrophobic moieties results in copolymers with lower values of LCST. In the case of ionic comonomers hydrophilic property of

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such introductions varies upon pH.[12,14-16]

Copolymers based on NIPAAm have been applied in biomedical field and biotechnology for drug carriers with targeting and controlled release effects.[12-14,16,17] surface modification, cell culture matrixes, enzyme modification, proteins separation, *etc*.

In this work we performed copolymerization of NIPAAm with hydrophilic *N*-vinylpyrrolidone (NVP) to vary the LCST and gelling properties of resulting copolymers. NVP was chosen as a comonomer due to well-known biocompatible non-toxic and non-immunogenetic properties of its homopolymer.[18] We assumed that the combination of NIPAAm with NVP at mutual copolymerization would give us a copolymer with suitable physico-chemical properties, variable thermal-sensitivity, and biocompatibility.

Experimental

Materials. *N*-isopropylacrylamide (NIPAAm) was purchased from Kohjin Co., Ltd (Japan), and was purified by triple dissolving in *n*-hexane at 40 °C under stirring followed by precipitation at cooling at room temperature. The resulting precipitate was filtered out and dried on open-air overnight and then under vacuum for 24 h. *N*-vinylpyrrolidone (NVP) and 2,2'-azobisisobutyronitryl (AIBN) were obtained from Aldrich Chem. Co. NVP was freed from stabilizer by twice vacuum distillation at continuous bubbling with N_2. AIBN was twice recrystallized in methanol/ethanol (1/1 v/v%), filtered and dried in vacuum. Methanol, ethanol and diethyl ether were distilled at atmospheric pressure. All other chemicals were analytical grade and were used as received. Distilled water with pH 7.0 was used in all experiments.

Methods. NIPAAm and NVP were mixed at different ratio (95 : 5, 90 : 10, 85 : 15, 80 : 20, 75 : 25 and 70 : 30 mol% in feed) in the presence of initiator AIBN (55×10^{-3} mol/L) in benzene (90 wt%), pored in glass tube, degassed with N_2 for 15 min and sealed. Copolymerization was carried out at 60 °C for 8 and 15 h. In course of copolymerization, opalescent precipitation of copolymer appeared. Upon completion of the reaction, the precipitate was dissolved in ethanol and over-precipitated in diethyl ether. The procedure was repeated twice. Copolymers obtained were dried under vacuum till constant weight. H^1 NMR analysis in D_2O was applied to determine the copolymer composition. The spectra were recorded on a Varian H^1 NMR spectrophotometer (200 MHz) at 25 °C.

Cloud point measurement of the copolymer aqueous solutions was performed on UV-vis spectrophotometer (UV-1601PC, Shimadzu). The temperature was raised from 10 to 90 °C in 1 °C increments per min. The transmittance was measured at 450 nm versus temperature. Cloud point was determined as a temperature at that transmittance was 50% from initial at 10 °C. Gel point was taken as a temperature at that polymer solution looses fluidity. Time required the polymer solution of certain volume to become opaque or gel at its transferring from 20 to 37 °C was measured by a stopwatch. The amount of water excluded at syneresis study was weighed on analytical balance and was expressed as a percentage from full amount of water. Swelling degree of gelled copolymers over gel point was determined as water content per 1 g of dry weight of the copolymer.

Results and Discussion

Synthesis of NIPAAm-NVP Copolymers. Linear copolymers containing *N*-isopropylacrylamide with *N*-vinylpyrrolidone ([NVP] = 5~30 mol% in feed) were synthesized by radical copolymerization. Yield of the copolymers is decreased with increase of NVP concentration in feed (Figure 1). At the lowest content of NVP the yield of the copolymers after 15 h reaches quite high value - over 90 wt% that is rare occurred for polymers. With concentration of NVP increasing up to 30 mol%, copolymer yield is diminished to approximately 50 wt%, however it remained high. The tendency of the yield to be diminished with NVP content in feed is stronger expressed if reaction time is shorter (8 h). The slope of the yield curve in that case is sharper, especially in range of high concentration of NVP. It means that NIPAAm is consumed on the earlier stages of conversion; its concentration in reaction mixture decreases with time and lower active NVP is involved into reaction of copolymerization more actively. It results in significant composition inhomogeneity of the copolymers. Low yield of copolymers with high concentration of NVP in feed can be explained by dilution of NIPAAm and decrease of the reaction rate, especially at lower conversion degree. Longer duration (over 15 h) of the synthesis reaction leads to cross-linking of copolymers. So

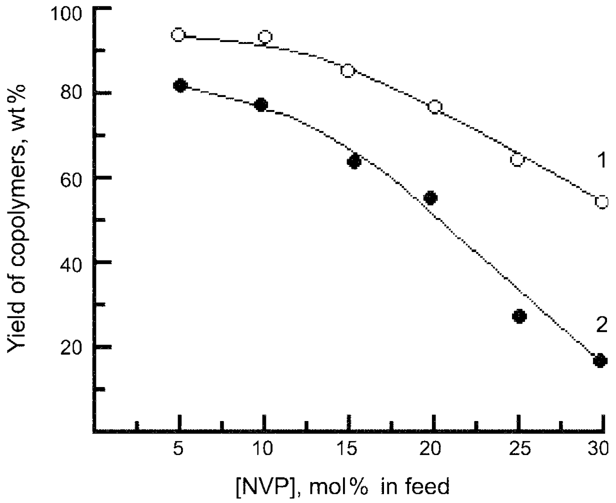

Figure 1. Yield of linear NIPAAm-NVP copolymers in dependence on feed composition. Synthesis conditions: 90 vol% of benzene, [AIBN] = $5{\cdot}10^{-3}$ mol/L; temperature 60 °C; duration of copolymerization: 1-15 h, 2-8 h.

that reaction time 15 h was considered as an optimal for radical synthesis of NIPAAm-NVP copolymers with high yield. Thus, these results of gravimetrical study are an evidence of lower activity of NVP in comparison with NIPAAm. At the maximum conversion in linear system the most active comonomer might be consumed almost completely, and polymerization rate of less active monomer tends to zero.[19,20]

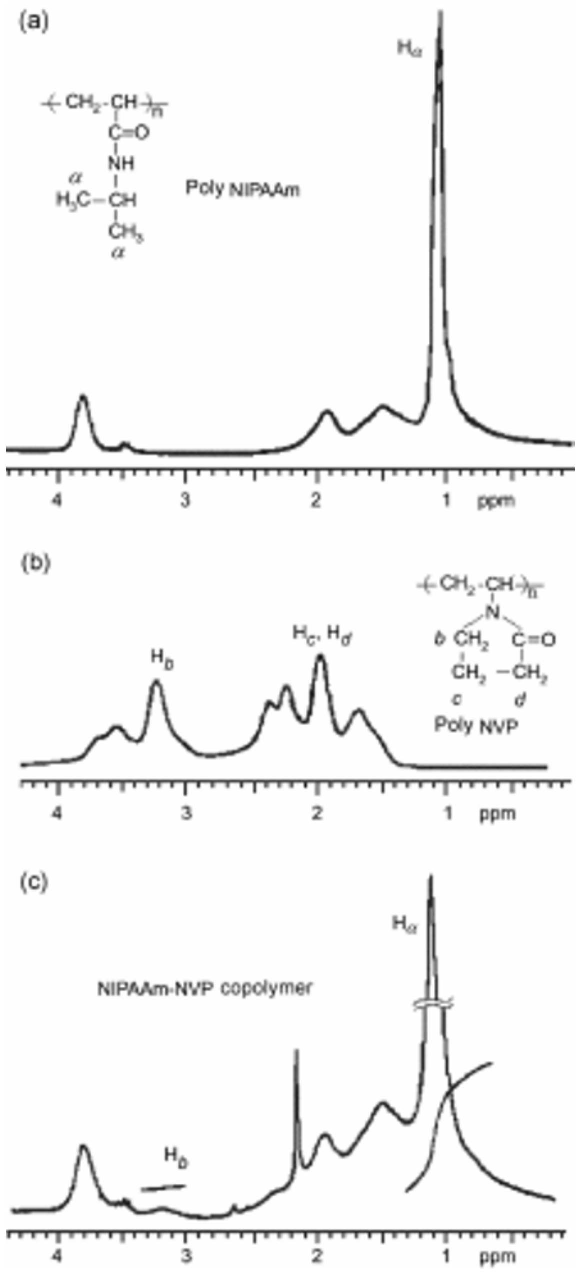

Figure 2. ^1H NMR spectra in D_2O of polyNIPAAm (a), polyNVP (b), and NIPAAm-NVP copolymer (c).

Table I. The Compositions of Feed and Copolymers of NIPAAm and NVP*

N	in Feed, mol%		in Copolymer, mol%	
	NIPAAm	NVP	NIPAAm	NVP
1	95	5	98.9	1.1
2	90	10	97.6	2.4
3	85	15	96.7	3.3
4	80	20	95.3	4.7
5	75	25	93.1	6.9
6	70	30	90.6	9.4

*Synthesized for 15 h.

The copolymer composition was measured by ^1H NMR analysis in D_2O. The presence of both NIPAAm and NVP in the copolymers was identified by a strong peak in the spectra assigned to protons in isopropyl group ($\delta = 1.12$ ppm), and relatively weak signal of protons in methylene group ($\delta = 3.17$ ppm) next to *N*-atom in *N*-vinylpyrrolidone (Figure 2). The copolymer composition was calculated as a ratio of the peak areas assigned to NIPAAm and NVP respectively taking into account the number of H-atoms, and is presented in Table I. As it is seen, the copolymers are enriched with NIPAAm monomer units in comparison with initial concentration of the monomers for all feed compositions studied. That also proves higher activity of NIPAAm in reaction of radical copolymerization with NVP. Low reactivity ratio for NVP was observed also at copolymerization with other acrylic comonomers such as glycidyl (2,3-epoxypropyl) methacrylate.[20]

Thermo-induced Phase Transition of the Copolymers. The NIPAAm-NVP copolymers exhibit thermosensitive behavior and they become opaque, and precipitate or form gel upon the temperature increase. Figure 3 presents the transmittance of polymer solutions upon temperature for copolymers with different ratio of NIPAAm and NVP. As it is seen, the transparency of the solutions is changed abruptly for copolymers of all compositions. It means that phase transition takes place upon heating, at that macromolecules transform from coil to compact globule conformation. This process occurs cooperatively due to destroying H-bonding between polymer and solvent and stabilization of globule conformation with inter- and intra-chain hydrophobic interactions. The more NIPAAm contain with hydrophobic isopropyl groups in copolymer structure the lower the temperature is required for copolymers to accept dense conformation.

The cloud point of 5 wt% solutions of the copolymers in distilled water (4 mL of volume) taken as a temperature regarding to 50%-transmittance from initial one, is increased with hydrophilic component concentration in the copolymers that confirms NVP incorporation in polymer structure (Figure 4). The cloud point range was varied from 33.5 to 36.3 °C for the lowest and highest NVP content respectively. However, the opaque solutions remain being free flowing with slightly reduced viscosity.

Time required the solutions to perform phase transition of

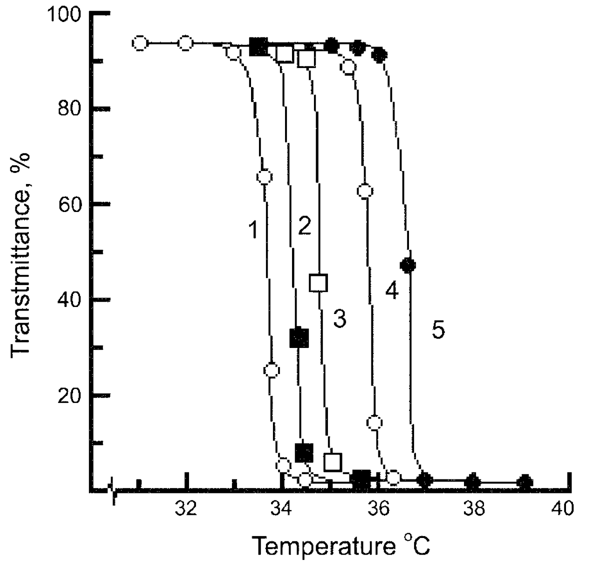

Figure 3. Transmittance of 5 wt% aqueous solutions on the temperature for NIPAAm-NVP copolymers at $\lambda = 450$ nm. [NIPAAm] : [NVP], mol% in feed: 1-98.9 : 1.1, 2-97.6 : 2.4, 3-96.7 : 3.3, 4-95.3 : 4.7, 5-93.1 : 6.9, 6-90.6 : 9.4.

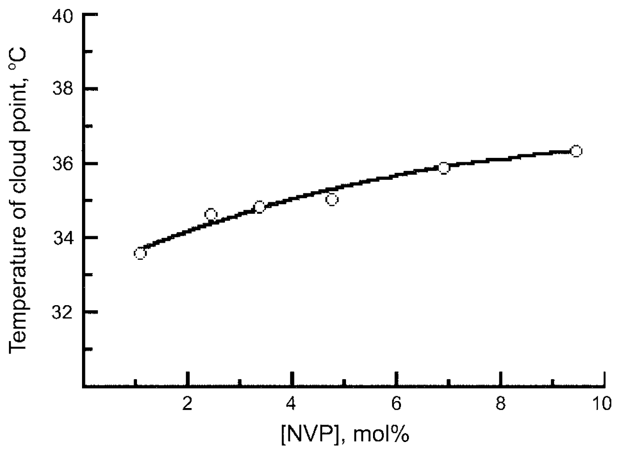

Figure 4. The influence of NIPAAm-NVP copolymer composition on their 5 wt% aqueous solutions cloud point.

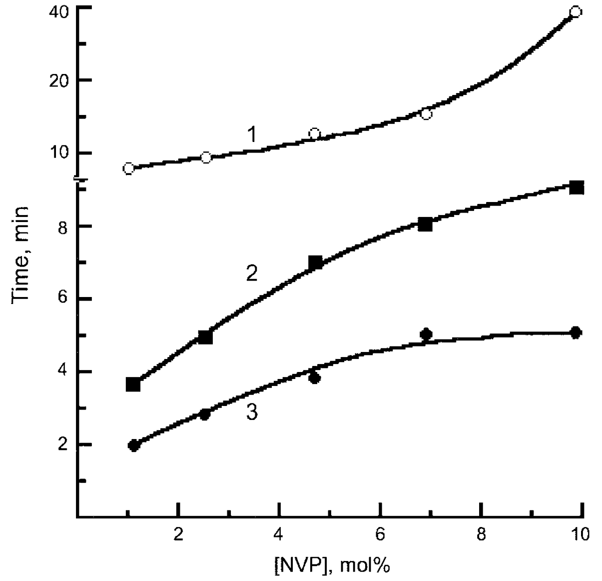

Figure 5. Time required aqueous solutions of NIPAAm-NVP copolymers (5 wt%, 4 mL) to reach cloud point at temperature: 1-37 °C, 2-45 °C, 3-50 °C.

the copolymers at certain temperature as they were immersed into water bath from ambient temperature, depends on copolymer composition. It decreases as the temperature and hydrophobic NIPAAm contents increase (Figure 5). At 37 °C for the copolymers relatively enriched by NVP content are characterized by one-order longer time than at 45 and 50 °C, and it is rapidly increased for the most hydrophilic sample.

The kinetics of thermo-induced phase transition was clearly also heat transfer-dependent, as larger volumes of precursor solution took longer time to gel under similar conditions. These results are obviously related to thermal induction time.[16] With further heating thermal-induced gelation occurred, resulting in lose fluidity of the solutions. Macromolecules in globule conformation aggregate probably with each other and lose their mobility at some certain temperature. Similar regularity was observed in experiments determining so-called gelling time, required the solutions to gel. However, the copolymers with NVP content 15 mol% and over do not form gel at 37 °C and stay liquid. The difference between cloud-point time and gelling time in case of [NIPAAm]: [NVP] = 98.9 : 1.1 mol% is varied from 5 min at 37 °C to 15 sec at 55 °C (data not shown). Thus, the gelation process is faster at higher temperature.

The phase diagram of "cloud point-concentration" for copolymers synthesized was obtained. It was found that dependence of cloud point value on the concentration of the copolymer solution (from 5 to 0.1 wt%) is quite negligible and varied within 1 or 2 degrees for all copolymer compositions range (data not shown). The same was observed for gel point, however solutions diluted lower 1 wt% does not form gel, and instead of the precipitation takes place.

Cloud and gel points of the copolymers decrease significantly more than 10 degrees with ionic strength increase from 0.01 to 1.0 due to salt-out effect typical for non-ionic polymers in aqueous solutions (Figure 6). In the presence of low molecular weight ions such as Na^+ and Cl^-, hydrogel bonds between polymer chains and water molecules become weaker that deteriorates thermodynamic quality of water as a solvent. That helps phase transition to take place at lower temperature. Thus, the copolymers with high content of hydrophilic NVP form gel easy even at low ionic strength (0.01), but not in distilled water as it was mentioned above.

The results observed demonstrate that cloud and gel points of NIPAAm-NVP copolymers can be carefully con-

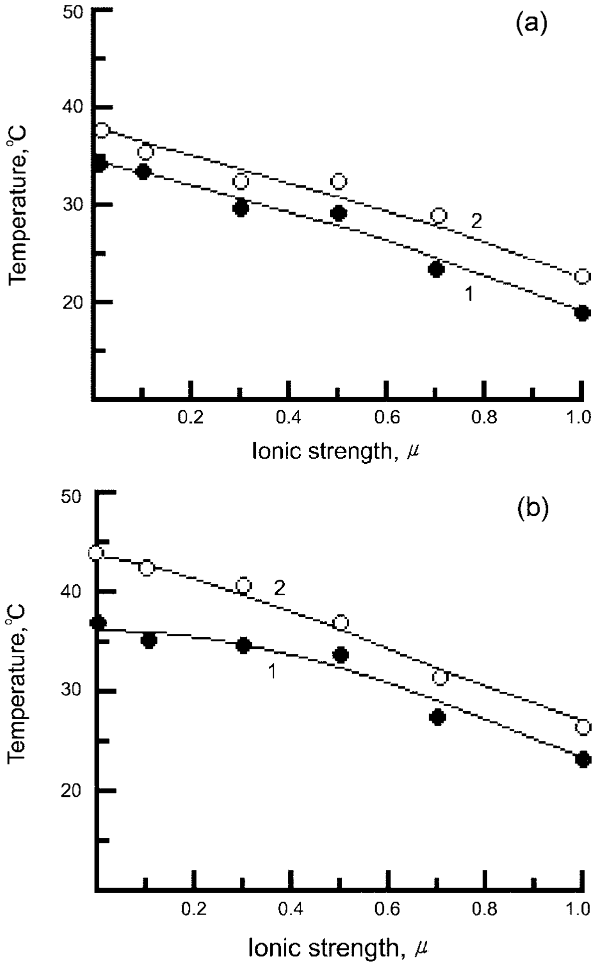

Figure 6. Effect of ionic strength on cloud point (1) and gelling point (2) of 5wt% aqueous solutions of [NIPAAm] : [NVP] = 97.6 : 2.4 mol% (a) and 90.6 : 9.4 mol% (b).

trolled by their composition and ionic strength of aqueous solutions, and concentration effect is less significant.

Swelling Characteristics of the Gelled Polymers. Thermo-gelling behavior of the copolymers was studied in order to find the most suitable sample to be used as an injectable implant material. At body temperature for the copolymers 98.9 : 1.1 and 97.6 : 2.4 mol% (5 wt% solution in distilled water) is accompanied by strong syneresis with time (Figure 7). That is swelling degree and consequently volume of the gels at constant temperature decrease with time while amount of water excluded increase. On molecular level, macrochains require some time to accept appropriate conformation at given conditions. After gelation occurred, polymer chains conformation undergoes slow reconstruction and contracts with time, expelling excess of water. However, further compacting is prevented by polymer elastic properties, and even quite compact globules contain large amount of water. It is necessary to note that gels safe the shape of glass vessel and contract in three dimensions simultaneously.

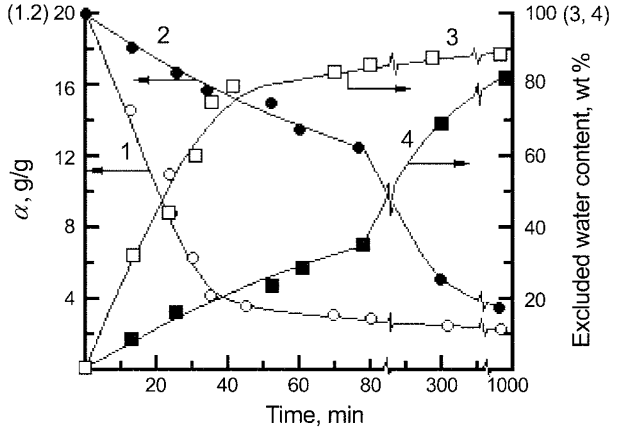

Figure 7. Kinetics of syneresis of NIPAAm-NVP copolymer solutions (5 wt%, 4 mL) at 37 °C after gelation. [NIPAAm] : [NVP], mol.%: 1,3-98.9 : 1.1; 2,4-97.6 : 2.4; 1,2-swelling degree of the gel; 3,4-excluded water in course of syneresis.

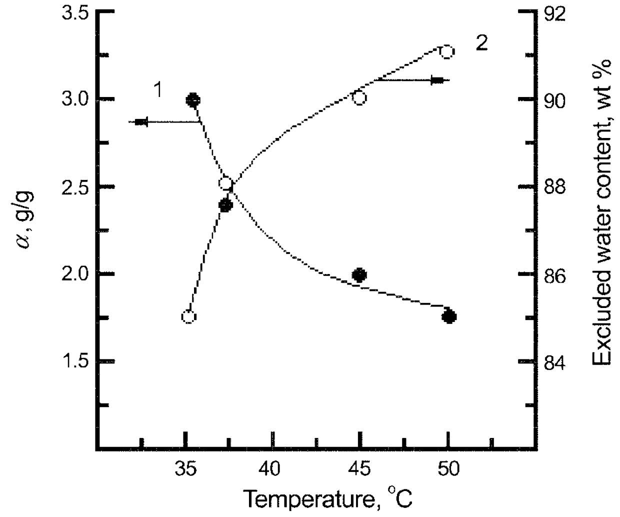

Figure 8. Influence of temperature on swelling degree (1) and amount of excluded water (2) after 24 h-long storage of [NIPAAm] : [NVP] = 98.9 : 1.1 mol% copolymer 5 wt% solutions gelled.

Preliminary mechanical tests showed tissue-like elasticity, so that after injection into a body a solution will turn to gel and the last one will not hurt surrounding tissues.

Syneresis is more sufficient for more hydrophobic copolymers enriched with NIPAAm units in their structure, as hydrophobic interactions play an important role in conformational changes. In our case the gels [NIPAAm] : [NVP] = 98.9 : 1.1 and 97.6 : 2.4 mol% contain 220 and almost 380% of water in collapsed state, respectively.

Syneresis occurs even stronger at higher temperature because of strengthening hydrophobic interaction within globules and expelling-off the water from them. For the copolymer with the most hydrophobic composition the swelling degree decreases from 300% at 36 °C to approximately 175% at 50 °C, and excluded water content is risen over 90 wt% from the initial one (Figure 8). Investigation of thermo-gelling behavior at increased ionic strength revealed the similar regularity at 37 °C (Figure 9). At continuous storage at 37 °C the copolymers gels shrink the stronger the higher concentration of low molecular weight salt. However this effect is more significant at low values of ionic strength (to 0.2). The amount of excluded water together with NaCl dissolved is changed antibately the swelling degree. The further increase of this parameter influences not so impressively. Table II contains the overview of data obtained for 4 samples of the copolymers with different composition in NaCl aqueous solutions. The more hydrophilic copolymers solutions in distilled water do not transfer to a gel state although they become opaque, as far as higher temperature is demanded for micelle aggregation. In 0.15 M concentration of NaCl imitating physiological solution, the only most hydrophilic copolymer does not gel. In more concentrated solutions all copolymers undergo thermo-gelling with progressive tendency. The kinetics of copolymer gel contraction is shown on Figure 10 as time profiles of swelling degree (a) and excluded water content (b). These parameters are changed rapidly during first 80 min as they are immersed in water bath at 37 °C, and then speed down approaching the limit values.

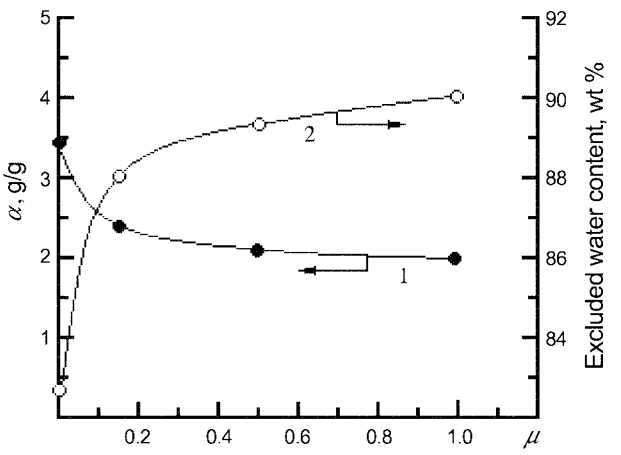

Figure 9. Influence of ionic strength on swelling degree (1) and amount of excluded water (2) after 24 h-long storage of [NIPAAm] : [NVP] = 97.6 : 2.4 mol% copolymer 5 wt% solutions gelled at 37 °C.

After 24 h storage at 37 °C of the hydrogels, their swelling

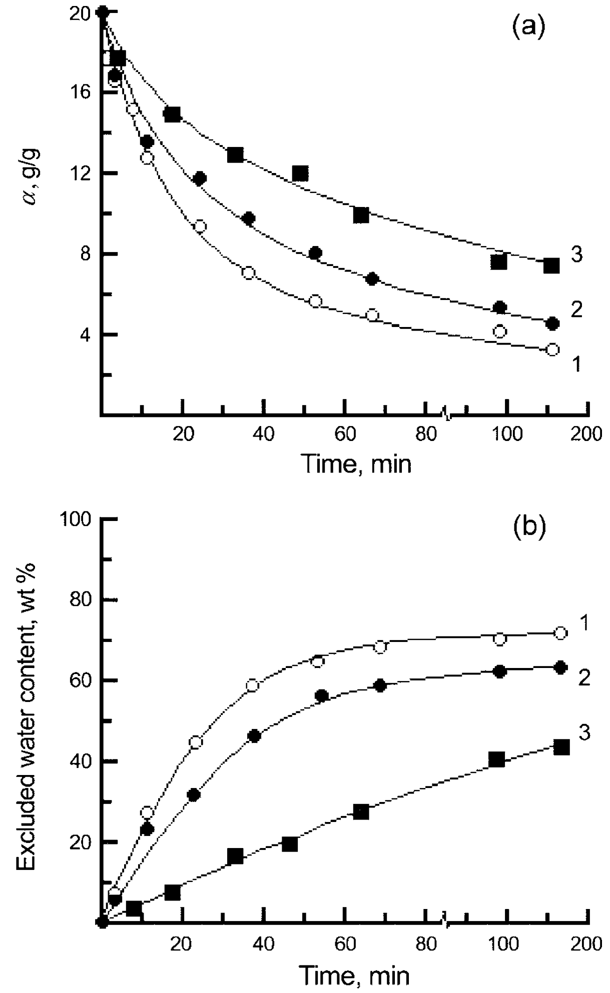

Figure 10. Swelling degree (a) and excluded water (b) time profile of NIPAAm-NVP copolymer solutions (5 wt%, 4 mL in 1 M NaCl) at 37 °C after gelation. [NIPAAm] : [NVP], mol%: 1-97.6 : 2.4, 2-95.3 : 4.7, 3-90.6 : 9.4.

Table II. Swelling Degree and Amount of Expelled Liquid at Syneresis of Thermo-gelled NIPAAm-NVP Copolymers*

[NIPAAm]:[NVP], mol% in feed	H_2O		NaCl					
			0.15 M		0.5 M		1.0 M	
	a	Liquid, wt%	a	Liquid, wt%	a	Liquid, wt%	a	Liquid, wt%
98.9:1.1	3.65	88.17	1.81	90.95	no data	no data	1.74	91.30
97.6:2.4	3.44	82.65	2.36	88.17	2.15	89.25	2.09	90.00
95.3:4.7	-	-	3.02	84.92	2.95	85.50	2.75	86.25
90.6:9.4	-	-	-	-	8.89	55.52	6.41	67.93

*Temperature 37 °C, concentration of initial aqueous solutions 5 wt%, volume 4 mL.

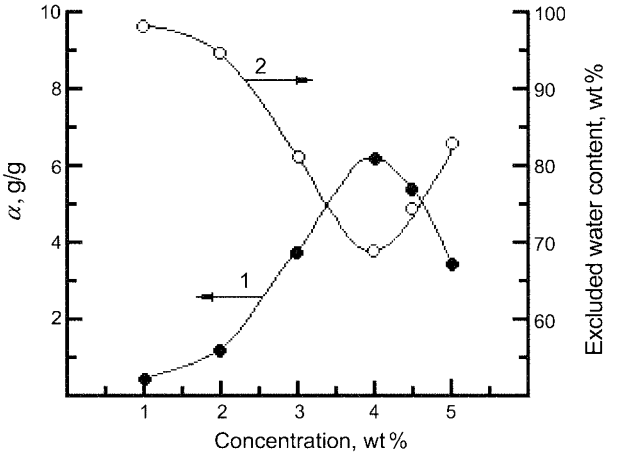

Figure 11. Influence of copolymer concentration on swelling degree (1) and amount of excluded water (2) after 24 h-long storage of the gelled 5 wt% solutions [NIPAAm] : [NVP] = 97.6 : 2.4 mol% in feed.

degree depends on concentration of initial solution and has an extreme character (Figure 11). Namely, gels formed from low-concentrated solutions show extremely low swelling degree. High-concentrated solutions also turn to quite collapse gels. Maximum swelling degree is shown for the solution with intermediate concentration (4 wt%) of the copolymer [NIPAAm] : [NVP] = 97.6 : 2.4 mol%. Probably, in diluted solutions water molecules separate macromolecules from each other. Upon heating they collapse independently, not disturbing one another to accept compact conformation. In that case physical gel is composed of dense compact globules, attracted by inter globule-globule interaction on their surface. As concentration increases, the macromolecules are spread more tightly in the solution, contact each other, and even penetrate into the space occupied by neighbor ones. In this condition compacting process occurs with some spatial difficulties, and macromolecules are not able to coil up completely. They form micelles contained more than one polymer chain what have bigger size. At that their aggregation carries out with large space between them. In macroscale it results in higher swollen gel. At high concentration macromolecules are in entangle state. Their contraction occurs like they are one entire thing, not leaving a big space for water molecules. Therefore, the swelling degree of resulted gel is decreased.

Conclusions

Radical copolymerization of NIPAAm with NVP results in the copolymers with variable hydrophilic/hydrophobic balance of macrochains ordered by feed composition. Gravimetric and H^1 NMR analysis showed lower activity of NVP in comparison with NIPAAm. The copolymers exhibit thermo-induced phase transition due to hydrophobic interactions between isopropyl groups. Rapid gelation in aqueous solutions was observed upon heating. Thermal sensitivity and gelation behavior of the copolymers can be regulated by the copolymer composition and environment parameters such as ionic strength and temperature. The gelation process is accompanied by contraction of networks formed, however resulting gels contain large amount of water. Samples with appropriate thermo-gelling properties are under investigation as an injectable implant material for plastic and functional surgery.

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